AMENDMENTS

In the Specification:

Please amend the paragraph on page 10, line 22 to page 11, line 3 as follows:

"Stringent conditions" or "high stringency conditions", as defined herein, may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, ~~such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride,~~ such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% FICOLL™/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 μg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium. citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C. FICOLL™ is a registered trademark for a synthetic sucrose polymer.

Please amend the paragraph on page 11, lines 14-22 as follows:

In the context of amino acid sequence comparisons, the term "identity" is used to express the percentage of amino acid residues at the same relative positions that are the same. Also in this context, the term "homology" is used to express the percentage of amino acid residues at the same relative positions that are either identical or are similar, using the conserved amino acid criteria of BLAST analysis, as is generally understood in the art. For example, % identity values may be generated by WU-BLAST-2 (Altschul et al., Methods in Enzymology, 266: 460-480

(1996): ~~http://blast.wustl/edu/blast/ README.html~~). Further details regarding amino acid substitutions, which are considered conservative under such criteria, are provided below.

Please insert the following paragraph after page 12, line 17 and before page 12, line 18:

Another embodiment is a pharmaceutical composition comprising a polynucleotide that encodes a SGP28 polypeptide, wherein the polynucleotide is selected from the group consisting of (a) a polynucleotide having the sequence as shown in Table 1 (SEQ ID NO: 2), wherein T can also be U; (b) a polynucleotide having the sequence as shown in Table 1 (SEQ ID NO: 2), from nucleotide residue number 3 through nucleotide residue number 776, wherein T can also be U; (c) a polynucleotide encoding a SGP28 protein having the amino acid sequence shown in Table 2 (SEQ ID NO: 3); (d) a polynucleotide that is a fragment of the polynucleotide of (a), (b) or (c) that is at least 20 nucleotide bases in length; (e) a polynucleotide that is fully complementary to a polynucleotide of any one of (a)-(d); or (f) a polynucleotide that selectively hybridizes under stringent conditions to the polynucleotide of any one of (a)-(d).

Please amend the paragraph on page 18, line 18, to page 19, line 3 as follows:

The SGP28 cDNA sequences described herein enable the isolation of other polynucleotides encoding SGP28 gene product(s), as well as the isolation of polynucleotides encoding SGP28 gene product homologues, alternatively spliced isoforms, allelic variants, and mutant forms of the SGP28 gene product. Various molecular cloning methods that can be employed to isolate full length cDNAs encoding a SGP28 gene are well known (See, for example, Sambrook, J. et al. Molecular Cloning: A Laboratory Manual, 2d edition., Cold Spring Harbor Press, New York, 1989; Current Protocols in Molecular Biology. Ausubel et al., Eds., Wiley and Sons, 995). For example, lambda phage cloning methodologies may be conveniently employed, using commercially available cloning systems ~~(e.g., Lambda ZAP Express, Stratagene).~~ (e.g., Lambda ZAP Express™, which are DNA vector kits consisting essentially of

vectors and reagents sold by STRATAGENE™). Phage clones containing SGP28 gene cDNAs may be identified by probing with labeled SGP28 cDNA or a fragment thereof. For example, in one embodiment, the SGP28 cDNA (Table 1; SEQ ID NO: 2) or a portion thereof can be synthesized and used as a probe to retrieve overlapping and full length cDNAs corresponding to a SGP28 gene. The SGP28 gene itself may be isolated by screening genomic DNA libraries, bacterial artificial chromosome libraries (BACs), yeast artificial chromosome libraries (YACs), and the like, with SGP28 DNA probes or primers.

Please amend the paragraph on page 19, line 24, to page 20, line 3 as follows:

A wide range of host vector systems suitable for the expression of SGP28 proteins or fragments thereof are available, see for example, Sambrook et al., 1989, supra; Current Protocols in Molecular Biology, 1995, supra). Preferred vectors for mammalian expression include but are not limited to pcDNA 3.1 myc-His-tag ~~(Invitrogen)~~ (INVITROGEN™) and the retroviral vector pSRαtkneo (Muller et al., 1991, MCB 11:1785). Using these expression vectors, SGP28 may be preferably expressed in several prostate cancer and non-prostate cell lines, including for example 293, 293T, rat-1, 3T3, PC-3, LNCaP and TsuPr1. The host vector systems of the invention are useful for the production of a SGP28 protein or fragment thereof. Such host-vector systems may be employed to study the functional properties of SGP28 and SGP28 mutations.

Please amend the paragraph on page 25, line 29, to page 26, line 5 as follows:

In a specific embodiment described in the examples that follow, a secreted form of SGP28 may be conveniently expressed in 293T cells transfected with a CMV-driven expression vector encoding SGP28 with a C-terminal 6XHis and MYC tag (pcDNA3.1/mycHIS, ~~Invitrogen~~ INVITROGEN™). The secreted HIS-tagged SGP28 in the culture media may be purified using a nickel column and standard techniques. Alternatively, an AP-tag system may be used. Various constructs for expression of SGP28 are described in the examples below.

Please amend the paragraph on page 31, lines 13-28 as follows:

A SGP28 antibody or fragment thereof of the invention may be labeled with a detectable marker or conjugated to a second molecule, such as a cytotoxin or other therapeutic agent, and used for targeting the second molecule to a SGP28 positive cell (Vitetta, E.S. et al., 1993, Immunotoxin therapy, in DeVita, Jr., V.T. et al., eds., Cancer: Principles and Practice of Oncology, 4th ed., J.B. Lippincott Co., Philadelphia, 2624-2636). Examples of cytotoxic agents include, but are not limited to ricin, ricin A-chain, doxorubicin, daunorubicin, ~~taxol,~~ TAXOL™ (an anticancer preparation), ethidium bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, dihydroxy anthracin dione, actinomycin, diphtheria toxin, Pseudomonas exotoxin (PE) A, PE40, abrin, abrin A chain, modeccin A chain, alpha-sarcin, gelonin, mitogellin, retstrictocin, phenomycin, enomycin, curicin, crotin, calicheamicin, sapaonaria officinalis inhibitor, and glucocorticoid and other chemotherapeutic agents, as well as radioisotopes such as ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y, and ^{186}Re. Suitable detectable markers include, but are not limited to, a radioisotope, a fluorescent compound, a bioluminescent compound, chemiluminescent compound, a metal chelator or an enzyme. Antibodies may also be conjugated to an anti-cancer pro-drug activating enzyme capable of converting the pro-drug to its active form. See, for example, US Patent No. 4,975,287.

Please amend the paragraph on page 46, lines 11 to 25 as follows:

Cancer immunotherapy using anti-SGP28 antibodies may follow the teachings generated from various approaches that have been successfully employed in the treatment of other types of cancer, including but not limited to colon cancer (Arlen et al., 1998, Crit. Rev. Immunol. 18:133-138), multiple myeloma (Ozaki et al., 1997, Blood 90:3179-3186; Tsunenari et al., 1997, Blood 90:2437-2444), gastric cancer (Kasprzyk et al., 1992, Cancer Res. 52:2771-2776), B-cell lymphoma (Funakoshi et al., 1996, J. Immunother. Emphasis Tumor Immunol. 19:93-101), leukemia (Zhong et al., 1996, Leuk. Res. 20:581-589), colorectal cancer (Moun et al., 1994, Cancer Res. 54:6160-6166); Velders et al., 1995, Cancer Res. 55:4398-4403), and breast cancer (Shepard et al., 1991, J. Clin. Immunol. 11:117-127). Some therapeutic approaches involve

conjugation of naked antibody to a toxin, such as the conjugation of ^{131}I to anti-CD20 antibodies ~~(e.g., Bexxar, Coulter Pharmaceutical),~~ (e.g., Bexxar™, Coulter Pharmaceutical™), while others involve co-administration of antibodies and other therapeutic agents, such as Herceptin™ (trastuzumab) with paclitaxel ~~(Genentech, Inc.).~~ (Genentech,™ Inc.). For treatment of prostate cancer, for example, SGP28 antibodies can be administered in conjunction with radiation, chemotherapy or hormone ablation.

Please amend the paragraph on page 54, line 25 to page 55, line 11 as follows:

In another embodiment, SGP28 nucleic acid molecules encoding specific cytotoxic T lymphocyte (CTL) epitopes may be employed. CTL epitopes can be determined using specific algorithms (e.g., Epimer, Brown University) to identify peptides within a SGP28 protein that are capable of optimally binding to specified HLA alleles. One suitable algorithm is the HLA Peptide Motif Search algorithm available at the Bioinformatics and Molecular Analysis Section (BIMAS) web site ~~(http://bimas.dcrt.nih.gov/)~~. This algorithm is based on binding of specific peptide sequences in the groove of HLA Class I molecules and specifically HLA-A2 (Falk et al., 1991, Nature 351:290-6; Hunt et al., 1992, Science 255:1261-3; Parker et al., 1992, J. Immunol. 149:3580-7; Parker et al., 1994, J. Immunol. 152:163-75). The HLA Peptide Motif Search algorithm allows location and ranking of 8-mer, 9-mer, and 10-mer peptides from a complete protein sequence for predicted binding to HLA-A2 as well as other Class I molecules. Most HLA-A2 binding peptides are 9-mers, favorably containing a leucine at position 2 and a valine or leucine at position 9 (Parker et al., 1992, J. Immunol. 149:3580-7). Actual binding of peptides to HLA-A2 can be evaluated by stabilization of HLA-A2 expression on the antigen processing defective cell line T2 (Xue et al., 1997, Prostate 30:73-8; Peshwa et al., 1998, Prostate 36:129-38). Immunogenicity of specific peptides can be evaluated *in vitro* by stimulation of CD8+ CTL in the presence of dendritic cells (Xue et al.; Peshwa et al., *supra*).

Please amend the paragraph on page 56, lines 15-24 as follows:

Genetic immunization methods may be employed to generate prophylactic or therapeutic humoral and cellular immune responses directed against cancer cells expressing SGP28. Constructs comprising DNA encoding a SGP28 protein/immunogen and appropriate regulatory sequences may be injected directly into muscle or skin of an individual, such that the cells of the muscle or skin take up the construct and express the encoded SGP28 protein/immunogen. Expression of the SGP28 protein immunogen results in the generation of prophylactic or therapeutic humoral and cellular immunity against prostate and other SGP28-expressing cancers. Various prophylactic and therapeutic genetic immunization techniques known in the art may be used ~~(for review, see information and references published at Internet address www.genweb.com)~~.

Please amend the paragraph on page 59, lines 6-10 as follows:

Tumor tissue and cell lines were homogenized in ~~Trizol reagent (Life Technologies, Gibco BRL)~~ TRIZOL® reagent (Life Technologies, Gibco BRL™; TRIZOL® reagent is a monophasic solution of phenol and guanidine isothiocyanate suitable for isolating total RNA, DNA, and proteins) using 10 ml/ g tissue or 10 ml/ 10^8 cells to isolate total RNA. Poly A RNA was purified from total RNA using ~~Qiagen's Oligotex~~ QIAGEN™'S OLIGOTEX™ mRNA Mini and Midi kits. Total and mRNA were quantified by spectrophotometric analysis (O.D. 260/280 nm) and analyzed by gel electrophoresis.

Please amend the paragraph on page 64, line 22 to page 65, line 4 as follows:

SGP28 mRNA expression in normal human tissues was first analyzed by northern blotting two multiple tissue blots obtained from ~~Clontech~~ CLONTECH™ (Palo Alto, California), comprising a total of 16 different normal human tissues, using labeled 36P1G3 cDNA as a probe (sequence as in Example 1). RNA samples were quantitatively normalized

with a β–actin probe. The results are shown in FIGS. 1A-B and indicate that, within the 16 tissues tested, the SGP28 gene is exclusively expression in prostate, testis and ovary. Interestingly, the prostate and ovary exhibit a 2.4 kb transcript, while testis expresses a 1.6 kb message (the 1.6 kb message could represent another SGP28 family member). The lower molecular weight signal in normal testis is probably due to cross-hybridization of the probe (SSH fragment) to CRISP2 message. An identical transcript is seen for CRISP2 on this normal panel using a gene specific oligonucleotide probe in the publication by Kratzschmar, J. et al., 1996, Eur. J. Biochem. 236:827-836.

Please amend the paragraph on page 66, line 27 to page 67, line 6 as follows:

The 36P1G3/SGP28 protein without the signal sequence (amino acids 33 to 258) was cloned into pAPtag-5 ~~(GenHunter Corp. Nashville, TN).~~ (GenHunter™ Corp. Nashville, TN). This construct generates an alkaline phosphatase fusion at the C-terminus of the 36P1G3 protein while fusing the IgGK signal sequence to N-terminus. The resulting recombinant 36P1G3 protein is optimized for secretion into the media of transfected mammalian cells and can be used to identify proteins such as ligands or receptors that interact with the 36P1G3 protein. Protein expression is driven from the CMV promoter and the recombinant protein also contains myc and six histidines fused to the C-terminus of alkaline phosphatase. The Zeosin resistance gene allows for selection of mammalian cells expressing the protein and the ampicillin resistance gene permits selection of the plasmid in E. coli.

Please amend the paragraph on page 72, lines 13-17 as follows:

SGP28-mediated effects may be assayed in cells showing mRNA expression. Luciferase reporter plasmids may be introduced by lipid mediated transfection ~~(TFX-50, Promega).~~ (TFX-50, Promega™). Luciferase activity, an indicator of relative transcriptional activity, is measured by incubation of cells extracts with luciferin substrate and luminescence of the reaction is monitored in a luminometer.

Please amend the paragraph on page 73, lines 5-14 as follows:

Cell lines expressing SGP28 can also be assayed for alteration of invasive and migratory properties by measuring passage of cells through a matrigel coated porous membrane chamber ~~(Becton Dickinson).~~ (Becton Dickinson™). Passage of cells through the membrane to the opposite side is monitored using a fluorescent assay (Becton Dickinson Technical Bulletin #428) using calcein-Am (Molecular Probes) loaded indicator cells. Cell lines analyzed include parental and SGP28 overexpressing PC3, 3T3 and LNCaP cells. To assay whether SGP28 has chemoattractant properties, parental indicator cells are monitored for passage through the porous membrane toward a gradient of SGP28 conditioned media compared to control media. This assay may also be used to qualify and quantify specific neutralization of the SGP28 induced effect by candidate cancer therapeutic compositions.

Please amend the paragraph on page 76, lines 5-19 as follows:

To identify SGP28 peptides predicted to bind to the human MHC class I molecule HLA-A2, the complete amino acid sequence of the SGP28 protein was entered into the HLA Peptide Motif Search algorithm found in the Bioinformatics and Molecular Analysis Section (BIMAS) Web site ~~(http://bimas.dcrt.nih.gov/)~~. The results of SGP28 predicted binding peptides are shown in Table 5. The top 10 ranking candidates are shown along with their location, the amino acid sequence of each specific peptide, and an estimated binding score. The binding score corresponds to the estimated half-time of dissociation of complexes containing the peptide at 37°C at pH 6.5. Peptides with the highest binding score (i.e., 999.9 for SGP28 peptide 2) are predicted to be the most tightly bound to HLA Class I on the cell surface and thus represent the best immunogenic targets for T-cell recognition. Actual binding of peptides to HLA-A2 can be evaluated by stabilization of HLA-A2 expression on the antigen-processing defective cell line T2 (Xue et al., 1997, Prostate 30:73-8; Peshwa et al., 1998, Prostate 36:129-38). Immunogenicity of specific peptides can be evaluated *in vitro* by stimulation of CD8+ cytotoxic T lymphocytes

(CTL) in the presence of dendritic cells (Xue et al., 1997, Prostate 30:73-8; Peshwa et al., 1998, Prostate 36:129-38).